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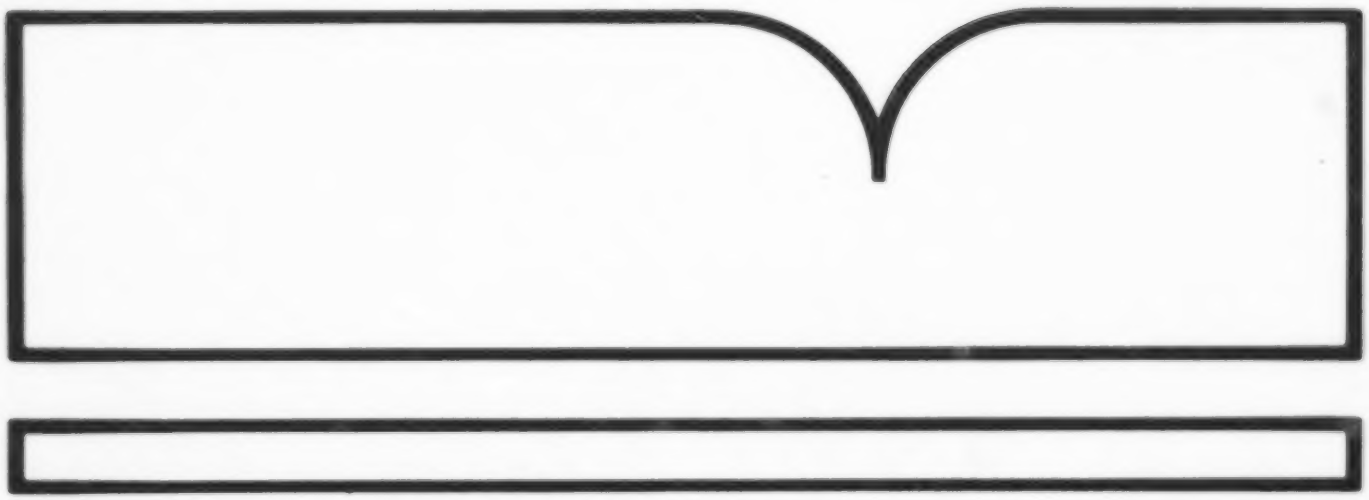

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16. ABSTRACT

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Pathways of glucose catabolism in the smut fungus *Ustilago violacea*

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Les sentiers cataboliques du glucose ont été étudiés chez des souches haploïdes et des souches diploïdes de *Ustilago violacea*, l'agent du charbon. Des études radiorespirométriques ont indiqué que les types copulateurs haploïdes de même que les souches diploïdes de ce basidiomycète ont tous catabolisé le glucose à la fois par le sentier d'Embden–Meyerhof et par celui du shunt hexose monophosphate. Toutefois, le sentier d'Entner–Doudoroff n'a pas été utilisé par l'une ou l'autre de ces souches. Les données radiorespirométriques ont d'autre part suggéré le fonctionnement d'un cycle d'acide tricarboxylique actif. Des tests enzymatiques *in vitro* ont établi la présence chez cet organisme de toutes les enzymes impliquées dans les sentiers opérateurs, ainsi que des enzymes reliées au cycle du glyoxylate. Les activités enzymatiques spécifiques au sentier d'Entner-Doudoroff n'ont pas été décelées. Aucune différence majeure n'a été décelée dans les sentiers de dissimilation du glucose entre les deux types copulateurs haploïdes ou entre les formes haploïdes et diploïdes de cet oganisme.

[Traduit par le journal]

The smut fungi are plant parasites of considerable economic import to agriculture (Fischer and Holten 1957; Westcott 1971). Research has focused primarily upon those smuts affecting cereal crops, such as *Ustilago maydis* (corn smut), *Tilletia caries* (wheat smut), and *Ustilago nuda* (barley smut). Studies of these organisms at the physiological and molecular levels have been rare but have included radiorespirometric and enzymatic analyses of carbon flow through the common pathways of glucose catabolism (Caltrider and Gottlieb 1963; McKinsey 1959; Newburgh and Cheldelin 1958, 1959). These key intermediary pathways have long been considered of potential importance in regulating morphogenetic changes in microbial cells (Goldman and Blumenthal 1963, 1964; Hammond 1977; Inderlied and Sypherd 1978). Some interesting correlations between cell morphology and pathway utilization have been noted in *T. caries* (Newburgh and Cheldelin 1958). The vegetative mycelium of this organism reportedly catabolizes glucose through a combination of the Embden–Meyerhof and hexose monophosphate shunt pathways, whereas the teliospores use the Entner–Doudoroff pathway exclusively. The latter observation places this organism together with the related basidiomycete *Caldariomyces fumago* into the select category of eukaryotic organisms with the ability to utilize the Entner–Doudoroff pathway (Ramachandran and Gottlieb 1963).

Learning more about this group of fascinating and economically important organisms at the molecular level may hold the key to controlling their destructive effects on cash crops and will certainly provide information about basic eukaryotic cell functions such as differential gene expression and cell morphogenesis. *Ustilago violacea* is a particularly advantageous organism to study in this regard because it not only displays a morphogenetic cycle associated with an alternation of generation, but it also possesses a parasexual cycle by which dikaryotic and diploid strains of free-living sporidia can be produced through fusion of opposite haploid mating types (Day and Jones 1968, 1969). The commonly studied *U. maydis* lacks a complete parasexual cycle (specifically, a means of inducing haploidization), which makes complete genetic analysis more difficult (Holliday 1961, 1964). It should prove possible to use *U. violacea* to study the effects of genome–genome interactions on gene expression and the potential expression of dominance and (or) complementation between co-resident haploid nuclei of differing lineage. This should allow a contribution to the body of knowledge already being accumulated on nuclear interactions in another basidiomycete, *Schizophillum commune* (De Vries and Reddingius 1984; Evers and Ross 1983; Hoge et al. 1982; Wang and Raper 1970).

The present work has examined the pathways of glucose catabolism in haploid and diploid strains of *U. violacea*. Using radiorespirometric and enymatic analyses it was shown that both haploid and diploid cells of *U. violacea* utilize glucose through the Embden–Meyerhof and hexose monophosphate shunt pathways. Neither cell form used the Entner–Doudoroff pathway. Further evidence suggested that all strains of the organism process acetate through the tricarboxylic acid cycle and the glyoxylate cycle.

Materials and methods

Organism and cultivation

Haploid mating types a_1 (ATCC 22000) and a_2 (ATCC 22001) of *Ustilago violacea* were isolated by Day and Jones (1968). Diploid strains of *U. violacea* were provided by E. D. Garber (University of Chicago) and were generated by crossing strain 2.21.74-11.1, which is a citrulline-requiring yellow strain derived from mating type a_1, with strain 7.12.73-15.12, which is a pyridoxine-requiring white strain

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derived from mating type a_2. Culture stocks were maintained on agar slants of the complete medium of Day and Jones (1968). Growth in liquid culture was started in the complete medium with shaking (250 rpm) at 22°C. The growing were subcultured twice from an inoculum of 2.4×10^6 cells/mL in the minimal medium (containing glucose, salts, and thiamine) of Day and Jones (1968) with shaking (250 rpm) at 22°C. All growth studies were performed on the second subculture in minimal medium. Radiorespirometric and enzymes studies were performed on cells taken from the mid-logarithmic phase of such cultures. Growth was monitored by measuring the culture turbidity at 500 nm and by microscopically counting cells in a Petroff–Hauser chamber.

Radiorespirometry

The pathways of glucose catabolism were determined by the method of Wang and co-workers (Wang et al. 1956; Wang et al. 1958; Wang 1972) which is based on the recovery of respiratory $^{14}CO_2$. The apparatus used in these experiments was similar to that described by Goldman and Blumenthal (1963). Radioactive carbon dioxide was recovered in 2-aminoethanol–2-methoxyethanol (1:2) and quantified by means of liquid-scintillation spectroscopy (Hash 1972). Glucose was measured by the anthrone assay (Seifter et al. 1950).

Enzyme assays

Cells were collected by centrifugation (10 000 × *g* for 15 min at 4°C), washed three times with 0.05 *M* sodium phosphate buffer (pH 7.0), and resuspended in ice-cold buffer appropriate for the specific assay to be conducted (see below). The cells were disrupted with an equal volume of glass beads (0.5 mm diameter) in a test tube (16 × 150 mm) agitated at top speed on a Lab-Line Supermixer (Lab-Line Industries, Melrose Park, IL) for 7 min. Following cell breakage additional cold buffer was added to the slurry of beads and cellular material, and after a thorough mixing, the cell-free fluid was decanted away from the beads. This solution was centrifuged at 8000 × *g* for 15 min at 4°C. The protein-rich supernatant fraction was collected with a Pasteur pipette, taking care to avoid the surface lipid-rich layer. Spectrophotometric enzyme assays were performed on these preparations according to the procedures cited in the appropriate tables below. Assays were performed in duplicate on a minimum of three independently prepared extracts. Protein in the cell-free extracts was measured by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Biochemical fractionation of cells

Cells incubated with D-[U-^{14}C]glucose for 1.5 relative time units (1 RTU = time required for complete consumption of administered glucose) were collected by centrifugation (6000 × *g* for 5 min at 4°C). The cell pellet was subjected in turn to extraction with (*i*) 5% (w/v) trichloroacetic acid at 2°C for 30 min, (*ii*) ethanol–ether (1:1) at 45°C for 30 min, and (*iii*) 5% (w/v) trichloroacetic acid at 100°C for 30 min. The radioactivity in each soluble fraction was assayed by liquid-scintillation spectroscopy (Hash 1972). Radioactivity in the hot trichloroacetic acid precipitate represented the difference between the sum of the aforementioned values and 100%.

Radioisotopes

D-[1-^{14}C]glucose, D-[2-^{14}C]glucose, D-[3-^{14}C]glucose, D-[3,4-^{14}C]glucose, D-[6-^{14}C]glucose, and D-[U-^{14}C]glucose were obtained from Volk Radiochemical Co., Skokie, IL.

Chemicals

All enzyme substrates, organic buffers, and other biochemicals were obtained from Sigma Chemical Co., St. Louis, MO. Inorganic salts were purchased from Mallinkrodt Chemical Works, St. Louis, MO. Organic solvents were obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. Dehydrated growth medium components were purchased from Difco Laboratories, Detroit, MI.

Results

Growth kinetics in minimal medium

Figure 1 represents the growth kinetics of *U. violacea* haploid mating types a_1 and a_2 and one of the diploid strains in the minimal medium. Doubling times were 11 and 10 h, respectively, for mating tyeps a_1 and a_2 and 17 h for the larger diploid strain. The increase in culture turbidity noted after the cells stopped dividing is probably attributable to an increase in the number of lipid vacuoles during the deceleration phase of growth, which we were able to observe by staining the vacuoles with Sudan black IV (Poon et al. 1974).

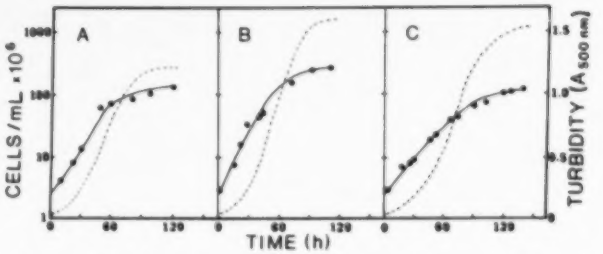

FIG. 1. Growth of *U. violacea* in minimal medium. Haploid and diploid strains of *U. violacea* were grown in the minimal medium of Day and Jones (1968) as described in the text. Growth was monitored by direct cell counts in a Petroff–Hauser chamber (●—●) and by the measurement of culture turbidity at 500 nm (------). (A) Haploid mating type a_1. (B) Haploid mating type a_2. (C) Diploid strain.

Radiorespirometry

Time-course recoveries of $^{14}CO_2$ from specifically labelled glucose are presented in Fig. 2 for mating types a_1 and a_2 and a typical diploid strain. All three strains exhibited similar patterns of recovery, indicating a rapid release of $^{14}CO_2$ from carbons 1 and 4 of the glucose molecule. The recovery of carbon 3 in the form of release CO_2 was relatively rapid but lower than that from carbons 1 or 4. The rate of recovery of carbon 2 was significantly lower than that of the aforementioned carbons but was faster than the rate of recovery of carbon 6 which was substantially lower than that of all other carbons. Cumulative recoveries of $^{14}CO_2$ from the specifically labelled glucose molecules are shown in the insets to Fig. 2, and indicate the preferential release of CO_2 from the 1, 3, and 4 positions of the pyranose ring during the course of 1 RTU.

Fate of assimilated glucose

After 1.5 RTUs the largest portion of the radioactivity from uniformly labelled glucose was found in the cold trichloroacetic acid soluble fraction of extracted cells, which contained mostly low molecular weight metabolites (Table 1). Significant amounts of radioisotope were also located in the respiratory CO_2 and in the cell suspension buffer (Table 1). The ether–ethanol fraction (which was composed primarily of lipids), the hot trichloroacetic acid fraction (which extracted primarily nucleic acids and polysaccharides), and the hot trichloroacetic acid precipitate (consisting mainly of proteins) accounted for significant portions of the initial radioisotope. The distribution of isotope among these chemical fractions did not vary appreciably as a function of strain.

Enzyme assays

To confirm the existence of pathways suggested by the radiorespirometric data, standard assays were performed for the enzymes that commonly participate in glucose catabolism. The enzymes of the Embden–Meyerhof pathway were found in extracts of all three strains (Table 2). With the exceptions of hexokinase and aldolase, these enzymes displayed only minor variations in specific activity with respect to strain.

The first two enzymes of the hexose monophosphate shunt,

TABLE 1. Distribution of ^{14}C in products of [U-^{14}C]glucose metabolism in *U. violacea* (as percent of total administered [^{14}C]glucose recovered)

Strain	Fraction					
	CO_2[a]	Buffer[b]	Cold TCA extract[c]	Ethanol–ether extract[d]	Hot TCA extract[e]	Hot TCA precipitate[f]
a_1	32.1	13.7	28.5	9.5	7.7	8.5
a_2	28.0	12.1	29.7	9.4	8.0	12.8
Diploid	33.1	13.9	28.2	9.8	7.9	7.2

[a]Metabolically generated CO_2 trapped in 2-aminoethanol – 2-methoxyethanol.
[b]Incubation solution remaining after sedimentation of cells by centrifugation.
[c]Material solubilized from cells following extraction with trichloroacetic acid at 2°C.
[d]Material solubilized from cold trichloroacetic acid extracted cells following extraction with ethanol–ether at 45°C.
[e]Material solubilized from ethanol–ether extracted cells following extraction with trichloroacetic acid at 100°C.
[f]Material remaining in precipitate following extraction with trichloroacetic acid at 100°C.

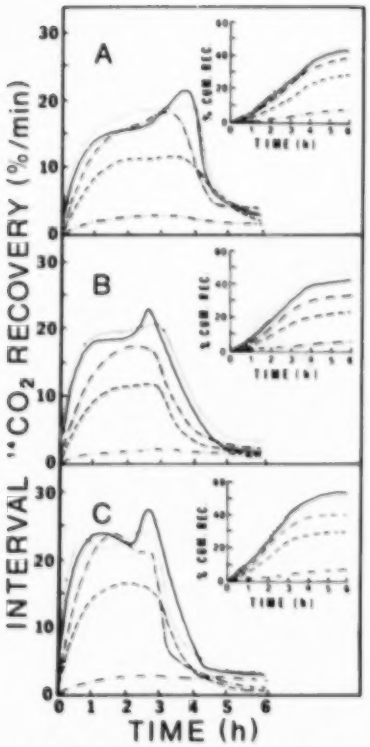

FIG. 2. Interval and cumulative recoveries of $^{14}CO_2$ produced from radiolabelled glucose by *U. violacea*. Interval recoveries are plotted on the main axes. Cumulative recoveries are represented in the insets. (A) Haploid mating type a_1; 1 RTU = 2.5 h. (B) Haploid mating type a_2; 1 RTU = 1.7 h. (C) Diploid strain; 1 RTU = 1.5 h. The recovery of $^{14}CO_2$ from D-[4-^{14}C]glucose was calculated from the recovery of D-[3-^{14}C]glucose and D-[3,4-^{14}C]glucose using the following formula: D-[4-^{14}C]glucose = 2 D-[3-^{14}C]glucose − D-[3,4-^{14}C]glucose. —, D-[1-^{14}C]glucose; ----, D-[2-^{14}C]glucose; —·—·—, D-[3-^{14}C]glucose; . . . ., D-[4-^{14}C]glucose; —· —· ·, D-[6-^{14}C]glucose.

glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were detected in extracts of all three strains of the organism (Table 3). The nonoxidative enzymes of this pathway were assayed and detected in extracts of the haploid mating type a_2 (Table 3). Ribulose-5-phosphate-3-epimerase was found to be very unstable in crude extracts, with the activity disappearing in 1 to 2 h after breakage of the cells. A combined assay for the enzymes of the Entner–Doudoroff pathway was performed with extracts of mating type a_2. No activity was detected (Table 3).

All enzymes of the tricarboxylic acid cycle were detected in extracts of all strains (Table 4). Significant differences in the specific activities of these enzymes with respect to strain occurred only in the cases of citrate synthetase, fumarase, and malate dehydrogenase. Malic enzyme was about half as active in extracts of diploid strains as in extracts of the haploid mating types (Table 4). NAD-specific isocitrate dehydrogenase could not be demonstrated in extracts of any of the strains. When NADPH was substituted for NADH in the malate dehydrogenase assay, no activity was observed; therefore, the presence of this enzyme in extracts did not affect the assay of malic enzyme.

The enzymes of the glyoxylate cycle, isocitrate layase and malate synthetase, were found in extracts of all strains examined (Table 4). Considerable fluctuations in specific activity with respect to strain were noted, especially in the case of malate synthetase.

Discussion

Analysis of interval recoveries of $^{14}CO_2$ from specifically labelled glucose permits quantitation of carbon flow through the commonly occurring catabolic pathways (Cheldelin 1961; Wang 1972). The Embden–Meyerhof pathway characteristically yields a rapid and equivalent release of $^{14}CO_2$ deriving from carbons three and four of the glucose molecule. Furthermore, equivalent yields are obtained from carbons two and five and from carbons one and six (Cheldelin 1961; Wang 1972). The Entner–Doudoroff pathway yields a quick and equivalent release of carbons one and four in the form of $^{14}CO_2$. A slower, equivalent release of carbons three and six as $^{14}CO_2$ would also be expected for this pathway (Cheldelin 1961; Wang 1972). The hexose monophosphate shunt is characterized by a rapid decarboxylation of glucose from the first position (Cheldelin 1961; Wang 1972). Data from all three strains of *U. violacea* examined here were indicative of active Embden–Meyerhof and hexose monophosphate shunt pahways. The calculated percent contributions of these pathways are presented for each strain in Table 5. The Entner–Doudoroff pathway was completely inactive in each case.

The above interpretations of the radiorespirometric data were supported by *in vitro* assays of the enzymes integral to the relevant pathways. All enzymes of the Embden–Meyerhof

TABLE 2. Specific activities of the enzymes of the Embden–Meyerhof pathway in cell-free extracts of *U. violacea*

Enzyme	Strain			Reference to assay procedure
	a_1 haploid	a_2 haploid	Diploid	
Hexokinase	0.136±0.025	0.186±0.015	0.0311±0.015	Anderson and Kamel 1966
Glucose-6-phosphate isomerase	1.071±0.057	0.93±0.09	1.29±0.061	Noltman 1966
Phosphofructokinase	0.0297±0.004	0.0252±0.006	0.0353±0.006	Vinuela et al. 1963; Sols and Salas 1966
Aldolase	0.0473±0.011	0.103±0.020	0.146±0.036	Rutter and Hunsley 1966
Glyceraldehyde-3-phosphate dehydrogenase	0.0197±0.005	0.023±0.0082	0.0167±0.0048	Krebs 1955
3-Phosphoglycerate kinase	0.0289±0.004	0.0356±0.0032	0.0239±0.0073	Bucher 1955*b*
2,3-Phosphoglycerate mutase	0.156±0.012	0.109±0.007	0.187±0.029	Sutherland et al. 1949
Enolase[a]	1550±205	1227±349	2103±185	Westhead 1966
Pyruvate kinase	0.918±0.14	0.847±0.20	1.04±0.21	Bucher 1955*a*
Pyruvate dehydrogenase complex	0.0474±0.014	0.0322±0.0078	0.0361±0.018	Amarasingham and Davis 1965

NOTE: Specific activities are defined as micromoles of substrate consumed or product formed per minute per milligram of protein. Standard deviations are included for all values.

[a] Specific activity for enolase is defined as Δ 0.001 $A_{232\ nm} \cdot min^{-1} \cdot mg\ protein^{-1}$.

TABLE 3. Specific activities of the enzymes of the hexose monophosphate shunt and Entner–Doudoroff pathways in cell-free extracts of *U. violacea*

Enzyme	Strain			Reference to assay procedure
	a_1 haploid	a_2 haploid	Diploid	
Glucose-6-phosphate dehydrogenase	0.334±0.027	0.399±0.020	0.326±0.046	Goldman and Blumenthal 1964
6-Phosphogluconate dehydrogenase	0.164±0.014	0.133±0.006	0.230±0.048	Goldman and Blumenthal 1964
Ribose-5-phosphate isomerase	NA[a]	0.117±0.012	NA	Novello and McLean 1968
Ribulose-5-phosphate-3-epimerase	NA	0.106±0.032	NA	Novello and McLean 1968
Transketolase	NA	0.186±0.048	NA	Novello and McLean 1968
Transaldolase	NA	0.161±0.039	NA	Novello and McLean 1968
Entner–Doudoroff combined assay	NA	<0.001	NA	Novello and McLean 1968

NOTE: Specific activities are defined as micromoles of substrate consumed or product formed per minute per milligram of protein. Standard deviations are included for all values.

[a] NA, no assay performed.

TABLE 4. Specific activities of the enzymes of the tricarboxylic acid cycle and the glyoxylate cycle in cell-free extracts of *U. violacea*

Enzyme	Strain			Reference to assay procedure
	a_1 haploid	a_2 haploid	Diploid	
Citrate synthetase	0.185±0.016	0.185±0.030	0.499±0.020	Dixon and Kornberg 1959
Aconitase[a]	208±29	231±13	150±29	Racker 1950
Isocitrate dehydrogenase (NADP linked)	0.0695±0.0014	0.075±0.0025	0.0736±0.0074	Kornberg 1955*a*
Isocitrate dehydrogenase (NAD linked)	<0.001	<0.001	<0.001	Kornberg 1955*b*
Succinate dehydrogenase	0.0046±0.0009	0.0036±0.001	0.0037±0.003	Arrigoni and Singer 1962
α-Ketoglutarate dehydrogenase	0.0054±0.0009	0.0031±0.001	0.00877±0.003	Amarasingham and Davis 1965
Fumarase	0.213±0.04	0.076±0.03	0.15±0.04	Racker 1950
Malate dehydrogenase (NAD linked)	4.40±0.097	4.27±0.26	1.44±0.34	Reeves et al. 1971
Malate dehydrogenase (NADP linked)	<0.001	<0.001	<0.001	Reeves et al. 1971
Malic enzyme	0.0241±0.005	0.0205±0.003	0.0092±0.006	Scrutton 1971
Malate synthetase	0.0423±0.006	0.00624±0.001	0.0298±0.007	Dixon and Kornberg 1959
Isocitrate lyase	0.011±0.002	0.0252±0.003	0.0157±0.001	Dixon and Kornberg 1959

NOTE: Specific activities are defined as micromoles of substrate consumed or product formed per minute per milligram of protein. Standard deviations are included for all values.

[a] Specific activity for aconitase is defined as Δ 0.001$A_{240\ nm} \cdot min^{-1} \cdot mg\ protein^{-1}$.

pathway were found in all strains of *U. violacea* that were examined. The occurrence of the hexose monophosphate shunt pathway in *U. violacea* was supported by the existence of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in all strains and the existence of the nonoxidative enzymes of this pathway in that strain of *U. violacea* which we examined (haploid mating type a_2). Presumably these enzymes would also be found in haploid mating type a_1 and in diploid strains if assayed. The failure to find activity of the two enzymes unique to the Entner–Doudoroff pathway in mating type a_2 supports the radiorespirometric data which indicates the absence of this pathway.

TABLE 5. Relative participation of glucose catabolic pathways in *U. violacea*

Strain	Percent participation[a]	
	Embden–Meyerhof pathway	Hexose monophosphate shunt
a_1 haploid	65.2	34.8
a_2 haploid	64.9	35.1
Diploid	57.6	42.4

[a]Based on the recovery of $^{14}CO_2$ from D-[1-^{14}C]glucose and D-[6-^{14}C]glucose after one RTU according to Wang (1972).

The recoveries of $^{14}CO_2$ from D-[2-^{14}C]glucose and D-[6-^{14}C]glucose provide information on activity of the tricarboxylic acid cycle. The significant recoveries of $^{14}CO_2$ from D-[2-^{14}C]glucose suggest the active operation of this pathway. It remains possible that the $^{14}CO_2$ recovery from D-[2-^{14}C]glucose was caused by a recycling of the fructose-6-phosphate formed in the hexose monophosphate shunt back through this same pathway. However, since the Embden–Meyerhof pathway is very active, this is probably not a significant source of $^{14}CO_2$ from D-[2-^{14}C]glucose. The recoveries of $^{14}CO_2$ from D-[6-^{14}C]glucose were lower than might be expected; however, this may be caused by removal of intermediates for biosynthetic purposes.

The functioning of the tricarboxylic acid cycle, suggested by the radiorespirometric data, is also supported by the *in vitro* detection of all the enzymes of this pathway in all the strains of *U. violacea* we examined. In addition, the enzymes of the glyoxylate cycle were found in substantial amounts in extracts of all three strains. Based upon relative enzyme activities, a significant amount of carbon may be routed through the glyoxylate cycle relative to the tricarboxylic acid cycle. Such a diversion of carbon through the glyoxylate cycle would, in fact, be necessitated if large amounts of glucose-derived intermediates were drained off as biosynthetic precursors as suggested by the low recoveries of $^{14}CO_2$ from D-[6-^{14}C]glucose noted above. Most of the assimilated catabolites of glucose noted above, especially those directed into protein and nucleic acid, would be tapped from the tricarboxylic acid cycle and would need replenishing through the glyoxylate cycle. Unfortunately, the relative participation of the glyoxylate cycle vis-a-vis the tricarboxylic acid cycle cannot be determined from the present radiorespirometric data.

Besides the tricarboxylic acid cycle or glyoxylate cycle, the most likely fate of acetate generated in the Embden–Meyerhof or hexose monophosphate shunt pathways would be in lipid synthesis. *Ustilago violacea* is known to accumulate substantial quantities of lipid (Poon et al. 1974), and after 1.5 RTU, 9.4–9.8% of the radioactivity from administered D-[U-^{14}C]glucose was incorporated into the lipid fraction of the cell (see above). After correction for radioisotope lost as CO_2 and for the percent of glucose converted into acetate, it was found that about one-quarter of the acetate was utilized for lipid synthesis. Metabolism of the remaining acetyl CoA by the tricarboxylic acid cycle can account for the amount of $^{14}CO_2$ released from D-[2-^{14}C]glucose without having to invoke recyclying of the hexoses through the hexose monophosphate shunt pathway.

Since *U. violacea* very actively respired for a significant period after the exhaustion of glucose from the incubation medium (Fig. 2), it appears that the organism takes in glucose faster than it can catabolize the sugar under the conditions used. This is a common phenomenon in many fungi (Wang 1972). More interesting was the fact that shortly after the exhaustion of glucose from the incubation medium significant increases in $^{14}CO_2$ recoveries were obtained from glucose molecules labelled in several different positions. This has frequently been observed and is often caused by activity of the tricarboxylic acid cycle (Wang 1972). The specific kinetics of radiolabelled carbon recovery make it unlikely that the tricarboxylic acid cycle is responsible in this case (Cheldelin 1961; Wang 1972). A more probable cause for the increased $^{14}CO_2$ recoveries would be that glucose accumulated intracellularly as a hexose pool or as a polymeric storage product such as starch. When the glucose was exhausted from the incubation medium, the reserves were catabolized resulting in the observed increased $^{14}CO_2$ recoveries.

Although the participation of the pathways of glucose catabolism did not differ significantly among the haploid and diploid strains examined, there were several enzymes whose activities varied markedly in different strains. Some of these differences can be accounted for by averaging the activities of the two mating types (e.g., fumarase, malate synthetase, and isocitrate lyase). However, the activities of other enzymes (e.g., citrate synthetase, malate dehydrogenase, malic enzyme, and hexokinase) in the diploid strains cannot be explained by the average of the activities of the two haploids or by the dominance of either mating type. Evidence has been presented that the mating type alleles of basidiomycetes may act as regulatory genes controlling the expression of a wide spectrum of structural genes (Ross et al. 1973; Wang and Raper 1970), although this evidence has recently been disputed (Evers and Ross 1983). Further examination of the noted enzymes of glucose catabolism in haploid and diploid strains of *U. violacea* may present an excellent opportunity to study the role of the mating type locus or the role of gene dosage (ploidy level) in the regulation of differential gene expression in basidiomycetes.

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